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PRODUCTION OF ANTIGENS AND ANTIBODIES FOR DIAGNOSIS OF ARBOVIRUS DISEASES

ANNUAL REPORT

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Production of antigens and antibodies for diagnosis of arbovirus diseases

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Antigens and sera were produced and standardized for use in the ELISA. Antigens were produced by sucrose-acetone extraction of suckling mouse brain for Oropouche, Bwamba, Tataguine, Dugbe, Hazara, Qalyub, Bandia, Hughes, Chagres, Mayaro, Bhanja, Japanese encephalitis, Bunyamwera, Ilheus, Ross River, and Germiston viruses. The antigens were inactivated with beta-propiolactone. Safety tests in suckling mice are not completed.

These same viruses were tested in the RK-13 rabbit kidney cell line adapted to grow in rabbit serum. Growth was obtained for Bunyamwera, Japanese encephalitis, Bwamba, Oropouche, Germiston, Bhanja, and Ilheus, but not for Qalyub, Ross River, and Chagres viruses on serial passage. Rabbits were immunized intravenously with the viruses which grew in RK-13 cells. Production of rabbit sera and testing of the antigens and antibody in the ELISA are not complete. C6/36 Aedes albopictus cells were adapted to grow in rabbit serum in anticipation of adaptation of the viruses which did not grow in RK-13 cells to this mosquito line.

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SUMMARY

Capture antibodies and antigens are needed for enzyme linked immunosorbent assay (ELISA) of human sera for antibody to arboviruses and other zoonotic viruses which are suspected of causing human disease in Latin America, Africa, and Asia. Antigens and sera were produced and standardized for use in the ELISA. Antigens were produced by sucrose-acetone extraction of suckling mouse brain for Oropouche, Bwamba, Tataguine, Dugbe, Hazara, Qalyub, Bandia, Hughes, Chagres, Mayaro, Bhanja, Japanese encephalitis, Bunyamwera, Ilheus, Ross River, and Germiston viruses. The antigens were inactivated with beta-propiolactone. Safety tests in suckling mice are not completed.

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FOREWORD

In conducting the research described in this report, the investigator adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

BODY OF REPORT

Production of mouse brain sucrose-acetone extracted antigens. The major effort during the first 12-month period has been production of sucrose-acetone extracted antigens from infected mouse brains. The following is the status of antigen production:

Antigen	Strain	Passage	Number of lots	Volume to date (ml)
Japanese Enc.	P-3	YARU sm4	10	278
Bunyamwera	Smithburn	sm49	12	269
Ilheus	Laemmert	sm40	9	192
Mayaro	TR 15537	sm13,Verol	8	183
Qalyub	EgAr 370	sm4	8	163
Ross River	AusT-48	sm11	11	256
Oropouche	TR 9760	sm7	11	241
Hazara	JC 280	sm10	1	19
Bhanja	IG 690	sm20	8	161
Germiston	SA AR1050	sm17/19/20	13	329
Tataguine	IPD A252	sm3	10	280
Bwamba	M 459	sm87	11	287
Bandia	IPD A611	sm8	6	166
Dugbe	IB Ar1792	sm13	1	9

Production of antibody to arboviruses in rabbits. RK-13 continuous line of rabbit kidney cells was obtained from the American Type Culture Collection and adapted to growth on rabbit serum. The cells grew well and did not show signs of toxicity.

The following viruses were inoculated onto RK-13 monolayers which were washed three times with PBS on initial passage. Some viruses were passaged serially in RK-13 cells. Cells were observed for CPE as well as virus titer when subcultured to infant mice, i.c.:

Virus	Passages in RK-13	CPE	Titer in mice
Bunyamwera	1	+	>4.5 log LD50/ml
	2	+	3.8 log LD50
Mayaro	1	+	<1.5 log LD50
	2	+	not done
Japanese Enc.	1	–	>4.5 log LD50
	2	–	3.0 log LD50
Bwamba	1	+	>4.5 log LD50
	2	+	4.0 log LD50
Ross River	1	+	3.0 log LD50
	2	–	<1.5 log LD50
Hazara	1	–	not done
Oropouche	1	+	>4.5 log LD50
	2	+	2.5 log LD50
Germiston	1	+	>4.5 log LD50
	2	+	>4.5 log LD50
Qalyub	1	–	2.5 log LD50
	2	–	<1.5 log LD50
Tataguine	1	–	not done
Bhanja	1	–	2.5 log LD50
	2	–	<1.5 log LD50
Dugbe	1	+	not done
Chagres	1	–	not done
	2	–	<1.5 log LD50
Ilheus	1	+	>4.5 log LD50
	2	–	3.0 log LD50

Attempts are in progress to immunize rabbits with the following viruses: Bunyamwera, Japanese encephalitis, Hazara, Bhanja, Ilheus, Qalyub, Bwamba, Oropouche, Germiston, and Ross River using infected RK-13 cell supernatant fluids, 1 ml intravenously each week for 3 weeks, then a single inoculation again after 1-2 months, then bleeding. The Bunyamwera rabbit serum titered >1:16,000 when used as capture antibody in ELISA. The other rabbit sera are not yet tested.

It was anticipated in the original proposal that some viruses would not replicate well in RK-13 cells. We have shown that the C6/36 Aedes albopictus cell line can be maintained with medium containing rabbit serum. The cells were grown in 10% fetal bovine serum, then transferred to a maintenance medium containing 3% rabbit serum. The mosquito cells were maintained one week in this medium. It is anticipated that most of the viruses which do not replicate in RK-13 cells can be propagated in C6/36 cells. Rabbits will next be immunized with those viruses, using infected C6/36 fluids.

Some arboviruses, especially the nairoviruses, may not replicate in either the RK-13 or C6/36 cells. If this is the case, these viruses will be propagated in Vero or CER cells. If titers are still not sufficient to immunize rabbits, it may be necessary to purify virus on gradients and inoculate rabbits with the purified fractions.